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Project Summary

The Stability and Mobility of Mutagenic Activity from Wastewater and Sludge in Agricultural Soils

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The objective of this research was to measure the fate and mobility of organic mutagens in municipal sludge amended soil. Initially, seventeen municipal sewage sludge samples were collected. The mutagenic potential of 49 separate fractions extracted from 17 samples of municipal sewage sludge was determined using the *Salmonella*/ microsome assay. None of the fractions extracted from four of the sludges induced a positive response in the bioassay. Twenty-six fractions failed to induce a doubling of revertant colonies at two consecutive dose levels, while seventeen fractions induced a moderate mutagenic response (35 to 100 net revertants 10 per milligrams), and six induced a relatively high mutagenic response (greater than 100 net revertants per 10 milligrams). The maximum specific activity was 187 net revertants per 10 mg induced by the methanol fraction extracted from the AD2 sludge with metabolic activation; while the maximum weighted activity was 1197 revertants per gram of sludge induced by the combined fraction of the SM4 sludge. For the sludge fractions tested, strain TA98 was the most sensitive, and the majority of fractions required metabolic activation to induce a positive response.

One sludge sample which induced a strong positive response was selected for use in a field study. Sludge samples were collected and applied at three application rates to undisturbed soils enclosed in lysimeters to monitor the fate and mobility of sludge borne mutagens in soil. The maximum specific activity of the sludge amended soil was 416 net revertants per 10 milligrams of residue induced by the methanol fraction extracted from the Wesvood soil collected 154 days following sludge application (totaling 150 Mg/ha). The mutagenic potential of the sludge amended soil was observed to decrease slowly with time. A positive response was still observed for several of the fractions extracted from the sludge amended soil collected 207 days after sludge application. The mutagenic activity per unit weight of soil decreased to near background levels over the course of the study for only one treatment (Padina sand with 50 Mg/ha). Eight of twenty-seven leachate samples from the waste amended soils induced a positive mutagenic response; while twelve samples induced a weighted activity of greater than 100 revertants per liter. The maximum specific activity of the acetone extractable organics recovered from the leachate water was 207 net revertants per 10 milligrams of residue induced without metabolic activation by the leachate water from the Wesvood soil which received two sludge applications totaling 150 Mg/ha.

None of the extracts from plants grown on the sludge amended soil induced a positive response in the bioassay. When the interactions of the Plant extracts were evaluated in the presence of positive controls or the extract of the sludge, synergistic, antagonistic, and additive interactions were observed. However, the extract of the plants grown

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on the sludge amended soil were observed to inhibit the mutagenic activity of the sludge extract.

The results indicate that municipal sludges may contain a persistent mutagenic residue. In addition, leaching of mutagens may be a serious problem in coarse textured soils, or in areas which receive high amounts of rainfall. Uptake of mutagens into plants does not appear to be a significant problem for the sludges, soils, plants, and application rates employed, as none of the extracts of plants grown on sludge amended soil induced a positive response.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back.)

Introduction

From the time man began to congregate in urban centers, the safe and economical disposal of municipal sewage has been a major concern. Given the present status of rising fertilizer costs and water shortages, it is of vital importance to our nation to use every effort to recycle the nutrients and water present in municipal sewage.

Large quantities of municipal sewage sludge are generated each day in the United States, much of which is ultimately disposed of by land application. Land treatment is designed to provide for the recycling of water and nutrients, and the retention or degradation of organics in the surface layer of soil. Health risks which may be associated with land application of sludge include pathogens (e.g., bacteria, viruses, protozoa and helminths), trace elements (e.g., cadmium, lead, and zinc), nitrates and organics. While much information exists pertaining to the environmental fate of specific pathogens, heavy metals, and nitrate, much less is known about the fate and composition of organic chemicals in municipal sewage.

A partial list of organic chemicals which have been identified in municipal wastewater or sludge includes a variety of chemical classes such as chlorinated solvents, polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons. Many of these chemicals have been found to induce mutagenic effects in short-term bioassays.

Care must be taken in the disposal of municipal sewage to prevent the transmission of mutagenic compounds to the human population. In a properly managed land treatment system, organic mutagens in a sludge amended soil may be transformed, degraded or immobilized. Under conditions of mismanagement or environmental stress, organic mutagens may be released from the facility by volatilization, leaching into groundwater, runoff into surface waters, or plant uptake. The present study was conducted to evaluate the fate of organic mutagens in a land application system.

Objectives

1. To evaluate the bacterial mutagenicity of the organic extractable fraction of municipal sludge from a variety of different sources.
2. To monitor the mobility and stability of these mutagens in municipal sludge amended soil.
3. To assess the potential for plant uptake of these mutagens from sludge amended soils.

Procedures

Seventeen municipal sludges were selected for evaluation to represent a range of domestic and industrial contributions. In addition, one treatment plant was selected from which multiple samples were collected over a ten month period to provide preliminary data on the variability of mutagenic constituents in sludge from a single source. Sludges were sequentially extracted using methylene chloride and methanol, and three fractions (methylene chloride, methanol and a combined fraction) were tested using the standard *Salmonella*/microsome assay.

To evaluate stability, sludge was applied on three separate occasions at two application rates to 18 undisturbed monolith lysimeters. Soil samples were collected from the sludge amended soil and control lysimeters immediately following and 28, 56, 116, 156, 175, and 210 days after sludge application. Soil samples were extracted with methylene chloride and methanol using the same procedures as were utilized for the sludge samples.

To evaluate mobility, leachate water was collected from suction cups installed 75 cm below the zone of incorporation. The leachate water was concentrated on a combined XAD2/XAD7 resin, and the adsorbed organics eluted with acetone. The residue from the leachate water was taken to dryness and redissolved in acetone for testing in the bioassay.

Finally, a limited study was conducted in the greenhouse to evaluate the potential for translocation of mutagens into alfalfa and bermudagrass grown on sludge amended soil. Bioassays were also conducted on mixtures of the plant extracts with known mutagens to evaluate potential interactions of plant constituents with mutagenic chemicals.

Results

The data from this study and that reported in the literature indicate that a tremendous variability can be anticipated between samples of sludge from different sources, as well as different samples of sludge from same source. In the present study, the specific activity of sludge fractions ranged from below detection to 187 net revertants per 10 mg of residue (approximately five times background), while, the weighted activity ranged from below detection to 1197 revertants per gram of sludge. For comparative purposes, a Weswood soil and a wood preserving waste induced a specific activity of 67 and 141 net revertants per milligram of residue, respectively; and, a weighted activity of 2 revertants per gram for the Weswood soil, and 20,070 revertants per gram for the wood preserving waste (Brown *et al.*, 1985 and Donnelly *et al.*, 1987). Thus, it appears that bioassays can serve as an indicator of the mutagenic activity of municipal sludges, although chemical analysis is an important tool to identify the components of active samples.

The data from this study showed that a minimum of eight weeks were required for the weighted activity of the soil to reach a maximum, and that the residual mutagens in the soil were persistent, and could still be detected at appreciable levels as long as 207 days following waste application. Three of the treatments, the Padina sand which received one application of 50 Mg/ha of sludge and the Weswood soil which received 50 Mg/ha or 100 Mg/ha of sludge, appeared to return to a weighted activity which was approximately equal to the weighted activity observed in the background soil samples during this time.

In the present study, appreciable quantities of mutagens were detected in several of the leachate samples. The specific activity of the leachate samples ranged from below detection to 207 revertants per 10 mg. Nine of twenty-six leachate samples induced a doubling of revertants at the highest dose tested. Two of the leachate samples collected from the lysimeters which received 150 Mg/ha of sludge induced a positive response. In addition, one of the samples collected from the Padina sand which received 50 Mg/ha induced a positive response. These data indicate that municipal sludge amended soils can release mutagens into the soil pore water (Brown et al., 1984).

The weighted activity of the leachate water from the soils best defines the quantitative bacterial mutagenicity of the leachate water. The weighted activities of the leachate samples collected from the sludge amended soils ranged from below detection to 416 revertants per liter. Twelve of twenty-seven samples induced a weighted activity of greater than 100 revertants per liter, with four of those samples inducing a weighted activity of greater than 300 revertants per liter. Thus, the weighted activity of the leachate samples was often more than twice that of drinking water, and occasionally greater than the weighted activity of surface water contaminated with a wood preserving waste.

For the sludges, soils, and plants employed for this study, there does not appear to be a significant potential for transmission of mutagens to the food chain through plants. The results indicate that at all dose levels tested, none of the extracts of plants grown on sludge amended soil induced a positive response in the Salmonella mutagenicity assay. Although the results indicate that mutagens were not detected in the plant extracts, this certainly does not indicate that plants will not adsorb mutagens from sewage sludge amended soils. The lack of mutagenic response may be due to either 1) the mutagens were not taken up by plants at levels which could be detected by the bioassays or 2) the mutagens were taken up by and into the plants, but plant constituents effectively inhibited the activity of the translocated mutagens.

The data from the literature and from the present study indicate that the potential of plant uptake of mutagens is limited. Additional information from the literature indicates that plant uptake will largely be governed by a chemical's water solubility, rate of biodegradation, and the clay content of the soil. Thus, the major health threat of plant uptake of mutagens is likely to be associated with the application of an unusually toxic sludge, unusually high concentrations of sludge, or sludge which had been weathered which may contain significant quantities of water soluble mutagens.

Conclusions

1. The bacterial mutagenicity of municipal sewage sludges exhibits tremendous variation both between different sources and from a single source.

2. The specific activity of several sludge fractions was not appreciably greater than the solvent control. For the mutagenic sludges, the maximum specific activity was 188 net revertants per 10 milligrams for the methanol fraction from the AD2 sludge with metabolic activation, and, the maximum weighted activity was 1197 net revertants per gram of sludge for the combined fraction of the SM4 sludge.

3. There does not appear to be a correlation between the level of mutagenicity in sludge, and the level of industrial contribution; however, this correlation may be more direct if it were possible to base this comparison on the specific type of industrial contribution.

4. Although a limited number of sludge fractions contained direct acting mutagens, for the majority of sludge fractions, mutagenicity was only observed in the presence of metabolic activation.

5. For the sludges and soils studied, the specific activity of the sludge amended soil increased with time following application, and required approximately six months to reach a maximum activity before exhibiting signs of detoxification. In addition, selected fractions of samples collected as long as 207 days following sludge application continued to induce a response that was greater than twice the concurrent solvent control.

6. For the majority of treatments evaluated, the weighted activity of the sludge amended soil was unchanged or slightly reduced for as long as 207 days following sludge application. However, for the fractions extracted from the Padina sand which received one sludge application at a rate of 50 Mg/ha, the weighted activity of the sludge amended soil collected 175 days after application was approximately equal to the weighted activity of the control soil.

7. Several samples of leachate water from the sludge amended soil were found to contain appreciable levels of organic mutagens. Although the chemical constituents of these samples were not identified, the results do indicate that mutagenic chemicals can migrate below the zone of sludge incorporation.

8. None of the extracts of plant samples tested induced a mutagenic response. Although these results suggest that translocation of mutagens did not occur, inhibition of mutagens by chlorophyll in the plants may have masked the activity of mutagenic chemicals which were present in the plant extracts.

Recommendations

1. Although bacterial mutagens were detected in most of the sludges tested, a more accurate description of the genotoxic potential of the municipal sludges would be obtained if additional testing were conducted using assays to measure DNA repair and chromosome damage, and these results were combined with a chemical analysis.

2. Short-term bioassays should be utilized as a tool for determining the persistence of mutagens in sludge amended soils.

3. The extraction procedure used for sludge and soil samples in this study is useful for environmental monitoring, however, a more detailed fractionation procedure would be valuable to isolate the most active mutagens in the sludge.

4. The rate of sludge application should be carefully monitored and regulated to prevent sludge born mutagens from leaching into the subsurface environment.

5. Additional research is needed to evaluate the interactions of climatic conditions and soil texture, especially as it affects degradation and leaching of mutagens in sludge amended soil.

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The complete report, entitled "The Stability and Mobility of Mutagenic Activity from Wastewater and Sludge in Agricultural Soils," (Order No. PB 88-237 243/AS; Cost: $25.95, subject to change) will be available only from:
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